Expression of a general transcription initiation factor, hTFIID, gene in normal human tissue—A quantitative assay for hTFIID mRNA based on polymerase chain reaction (PCR)—

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SUMMARY

A general transcription factor IID (TFIID) binds to the TATA box promoter element and regulates the expression of most eukaryotic genes transcribed by RNA polymerase II (Pol II). A highly sensitive, specific and quantitative assay for human TFIID (hTFIID) mRNA was developed based on polymerase chain reaction (PCR). The distinctive points of our procedure include the use of small amount of total cellular RNA (1 µg), a random primer for cDNA synthesis, β2-microglobulin (β2M) as an internal control and calculation of the relative value of hTFIID transcript from 32P-incorporation of the co-amplified PCR at different cycles. By this procedure, distribution of the hTFIID gene expression was for the first time demonstrated in normal human tissues and the amount of hTFIID mRNA was measured. In some tissues such as liver, fetal lung and placenta, moderate levels of hTFIID mRNA were detected. hTFIID transcript appeared correlated to total mRNA initiation and protein synthesis in tissue. This quantitative PCR procedure can be applied to more extensive studies of gene expression.

Key words: human TFIID, quantitative PCR assay, gene expression.

INTRODUCTION

Initiation of transcription in eukaryotes by RNA polymerase II (Pol II) is a complex process with the orchestrated function of several factors. The general transcription factors are thought to form a complex with Pol II near the transcriptional initiation site by interacting with common core-promoter elements.1,2) The most common core-promoter element in protein-coding genes is the TATA sequence (TATA box) typically located 25 to 30 base pairs (bp) upstream the transcriptional initiation site or initiation element in many Pol II genes. Transcriptional initiation requires several general transcription factors such as TFIID, TFIIA, TFIIB and TFIIE/F, and Pol II.1) TFIID binds specifically to the TATA box so that other general factors and Pol II assemble to form an active transcription complex at the promoter site. This factor may possibly be a target for several upstream gene specific transcription factors.2) TFIID is thus essential to the initiation of eukaryotic mRNA synthesis.

Because it has been difficult to purify, the mam-
malian TFIID has been poorly characterized. Recently, TFIID genes have been cloned from HeLa cells,\(^3\) of Drosophila,\(^3,5\) Arabidopsis\(^6\) and yeast\(^7\) by polymerase chain reaction (PCR). However, the distribution of human TFIID (hTFIID) in normal human tissue has yet to be examined.

In this study, a quantitative PCR procedure was developed to assay hTFIID mRNA and we examined the expression of the hTFIID gene in normal human tissues. The procedure and the expression in human tissues are discussed in the following.

**MATERIALS AND METHODS**

1. **Tissue samples and cell line**

A normal term placenta was obtained from a case of normal labor. Other normal human materials were obtained from postmortem tissue. An established human small cell lung cancer (SCLC) cell line, Lu 134 BS,\(^10\) was cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS). The cell line was provided by Dr. T. Terasaki. Tissue samples and cell harvest were frozen in liquid nitrogen and stored at -80°C until use.

2. **Oligonucleotides for amplification**

Oligonucleotides of 5'-primers, 3'-primers and probes were synthesized on an Applied Biosystems model 381 A synthesizer. hTFIID specific sequences were amplified using the following primers and probe.\(^3\)–\(^5\)

- **sense-strand primer-1 (P-1),**
  \[5'\gt\text{GCTGCAGCGGTTCAGCAGTC}<3'\]
  (residues 295–314)

- **antisense-strand primer-2 (P-2),**
  \[5'\gt\text{GCTCTGACTTTAGCACCTGT}<3'\]
  (residues 937–956)

- **antisense-strand probe,**
  \[5'\gt\text{AACTCTGGCTCATAACTACT}<3'\]
  (residues 841–860)

For quantitative co-amplification, \(\beta_2\)-microglobulin (\(\beta_2\)M) mRNA was selected as the internal control. The following primers and probe of \(\beta_2\)M were used.\(^11\)

- **Primer-1,**
  \[5'\gt\text{TCTTGGCTGGAGGGCATCC}<3',\]
  925–946 (exon I)

- **Primer-2,**
  \[5'\gt\text{ACACGGCAGGCATACTC}<3',\]
  1559–1579 (exon II)

PCR with these hTFIID and \(\beta_2\)M primers yielded 662 base pairs (bp) and 340 bp products, respectively.

3. **RNA preparation and cDNA synthesis**

Total cellular RNA was extracted by the guanidinium/hot phenol method.\(^12\) Single stranded cDNA was synthesized with 1 \(\mu\)g of total cellular RNA and 300 ng of random hexadeoxynucleotide primer (TAKARA) in 50 mm Tris-HCl (pH 8.3), 10 mm MgCl\(_2\), 100 mm KCl, 10 mm dithiothreitol, 1 mm dNTP, 25 units of human placental ribonuclease inhibitor (TAKARA) and 16 units of Rous associated virus 2 reverse transcriptase (TAKARA) in a total vol. of 20 \(\mu\)l. A reverse transcriptase reaction mixture was incubated at 42°C for 60 min and stored at -20°C until use. The efficiency of cDNA synthesis was assessed by \(^33\)P-incorporation as 20–30%.

4. **PCR**

PCR was conducted using a 10 \(\mu\)l aliquot of the synthesized cDNA mixture at a final concentration of 10 mm Tris-HCl (pH 8.8), 50 mm KCl, 2.5 mm MgCl\(_2\), 0.1 mg/ml gelatin, 200 \(\mu\)M each dNTP, 0.5 \(\mu\)M each 5' and 3' primers and 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer/Cetus) in final vol. of 50 \(\mu\)l. The mixture was overlaid with mineral oil and amplified with the Program Temp Control System PC-700 (ASTEC). Amplification was carried out for 25 cycles, each including denaturation at 97°C for 1 min, primer annealing at 56°C for 1 min and extension at 72°C for 3 min. To check the efficiency of the procedure and calculate relative amounts of transcripts, hTFIID and \(\beta_2\)M transcripts were co-amplified using the primer mixture in the same tube.
5. Agarose gel electrophoresis and southern blot

The PCR products electrophoresed in a 2% agarose gel with 1× TBE buffer (0.089 M Tris-borate, 0.089 M boric acid and 0.002 M EDTA). The gel was stained with 1 µg/ml ethidium bromide and photographed under UV-irradiation using Kodak Type 55 Polaroid film. The PCR products were transferred to a nylon membrane (Hybond-N, Amersham) and hybridized with the respective 5'-end labeled probes as previously described.\(^{12}\)

6. Quantitative analysis

cDNAs from two different tissues, Lu 134 BS and term placenta, were used for quantitative amplification by the incorporation of [\(\alpha\text{-}\)\(^{32}\)P]dCTP. 10 µCi (~110 TBq/mmol) of [\(\alpha\text{-}\)\(^{32}\)P]dCTP were added to 50 µl of each PCR reaction mixture and each was divided into five aliquots. 14 to 30 cycles of the same amplification were then carried out. After 2% agarose gel electrophoresis, ethidium bromide staining and obtaining a photogram, the gel was dried and exposed to X-P film. Appropriate bands were cut out from the gel and radioactivity was determined by Cerenkov counting. The amount of radioactivity was plotted on a semi-logarithmic graph against the number of PCR cycles.

In other human tissues, \(^{32}\)P-incorporation of co-amplified products at 25 cycles was determined as the average of triplicate measurements. Several negative control reactions were conducted in each experiment. Some negative controls contained water instead of reverse transcriptase or cDNA. A negative control without reverse transcriptase helped confirm DNA contamination.

RESULTS

To quantify the co-amplified transcripts, PCR by \(^{32}\)P-incorporation at 14 to 30 cycles was conducted using cDNA samples from two different tissues, Lu 134 BS and term placenta. 2% agarose gel electrophorogram with ethidium bromide staining and autoradiograms of the incorporation of Lu 134

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Fig. 1. Quantification of co-amplified PCR for the hTFIID and \(\beta\text{-}2\text{M}\) transcripts.

a: Ethidium bromide stained agarose gel electrophorogram of co-amplified PCR at different cycles in Lu134BS. A photogram of Polaroid film is shown. b: Autoradiograms of co-amplified PCR by \(^{32}\)P-incorporation at different cycles in Lu134BS. Amplified hTFIID and \(\beta\text{-}2\text{M}\) specific products for increasing number of cycles, \(n\), are shown. Exposure time was 1.0 h. c: Semi-logarithmic plot of \(^{32}\)P-incorporated PCR in Lu134 BS and term placenta. The extent of amplified hTFIID and \(\beta\text{-}2\text{M}\) transcripts was measured by Cerenkov counting of \(^{32}\)P-incorporation into the fragments visualized in b. ○ and △ indicated the amounts of hTFIID products from Lu134BS and placenta, respectively, and ● and ▲ \(\beta\text{-}2\text{M}\) from Lu134BS and placenta.
BS are shown in Figs. 1a and b. Figure 1a shows this procedure to have successfully co-amplified both hTFIID and β2M transcripts from Lu 134 BS. The 662 bp product of hTFIID transcript and 340 bp of β2M transcript were shown to be formed. Increase in the amount of co-amplified products of hTFIID and β2M was correlated to the number of cycles. Based on these amounts of Lu 134 BS and term placenta (data not shown), a semi-logarithmic plot was made (Fig. 1c). The count of each product was exponential for 14 to 26 cycles and then decreased slowly at 30 cycles. Some amplified bands due to artifacts were found beyond 30 cycles. Using the same specific activity of [α-32P] dCTP, the amount of the β2M transcript in two tissues was almost the same while that of the hTFIID transcript varied greatly between Lu 134 BS and placenta. The parallel increase of the hTFIID transcripts in these tissues was found at 14 to 26 cycles.

In PCR, amplification (Y) is determined from $Y = A(1 + R)^n$, where A is the initial amount of material, R the efficiency and n, the number of cycles. Y can be estimated from 32P-incorporation into the amplified bands and R deduced from the slope of the semi-log plot, $\log Y = \log A + n \log(1 + R)$. The absolute value of hTFIID mRNA ($A_{hTFIID}$) and β2M mRNA internal standard ($A_{β2M}$) cannot be determined directly. The relative value of $A_{hTFIID}$ was calculated from $A_{hTFIID}/A_{β2M} = Y_{hTFIID}/(Y_{β2M})(1 + R_{β2M})^n$.13 The absolute value of $A_{hTFIID}$ was calculated from $A_{hTFIID}/A_{β2M} = Y_{hTFIID}/(Y_{β2M})(1 + R_{β2M})^n$ based on the equations $Y_{hTFIID} = A_{hTFIID}(1 + R_{β2M})^n$ and $Y_{β2M} = A_{β2M}(1 + R_{β2M})^n$.13

At 14 to 26 cycles, transcript efficiency and values were calculated. In triplicate runs of Lu 134 BS co-amplification, $R_{hTFIID}$ was estimated as 48–49%, $R_{β2M}$ as 37–38% and $A_{hTFIID}/A_{β2M}$, as about 1/22–1/25 (4–4.5%). The estimated efficiency of hTFIID and β2M (14–26 cycles) was also constant in placenta, $R_{hTFIID}$ 48±1% and $R_{β2M}$ 38±2%. $A_{hTFIID}/A_{β2M}$ of placenta was calculated as 1.4–2.0%.

The same co-amplification PCR procedure at 25 cycles in exponential efficiency was performed to detect hTFIID expression in human tissues. The results of normal human tissue are shown in Fig. 2. The β2M transcript was ubiquitously expressed throughout all tissues while the hTFIID transcript at 662 bp varied greatly according to the tissues. Compared with the hTFIID transcript in Lu 134 BS, moderate levels of hTFIID-amplified products were found in the liver, kidney, fetal lung, placenta.

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![Fig. 2. Distribution of hTFIID transcript in human tissue.](image)

a: Ethidium bromide stained 2% agarose electrophorogram of co-amplification PCR products. 662 bp of hTFIID and 340 bp of β2M transcripts were specifically amplified. Lu134BS RT (−) indicates co-amplified PCR from Lu134BS cDNA sample without reverse transcriptase. cDNA was synthesized using total cellular RNA (1.0 μg) from various tissues and 25 cycles of PCR were conducted. b: Southern blot analysis of co-amplification PCR with the respective hTFIID and β2M probes. The probes and exposure time are indicated.
and adrenal gland. Low levels of hTFIID mRNA were expressed in the cerebral cortex, heart and spleen. In the cerebellum and adult lung, very low levels were found for longer exposure times. The hTFIID transcript was expressed to a greater extent in the SCLC cell line, Lu 134 BS, than in other normal human tissues. Neither hTFIID nor the β2M sequence could be amplified in the PCR reaction of Lu 134 BS cDNA without reverse transcriptase (Lu 134 BS, RT(−)). Chance amplification of contaminating DNA is thus ruled out in the procedure though the gene structure of hTFIID has yet to be clarified.

In this PCR co-amplification, the amount of the β2M transcript was expressed in all tissues and Lu 134 BS cell line. The amount of β2M mRNA has been shown to be <1.0% of the total mRNA in adult tissues.14) The estimated efficiencies, for R\textsubscript{hTFIID} and R\textsubscript{β2M}, were deduced as 48% and 38%, respectively. Based on these efficiencies and counts, \textsuperscript{32}P-incorporation of each hTFIID and β2M transcripts at 25 cycles, the relative amount of hTFIID mRNA to total mRNA was determined in various tissues. Estimations of hTFIID mRNA in different samples are shown in Table 1. Low levels of expression, 1−25\times10\textsuperscript{-5}% to total mRNA, were found in normal human tissues.

DISCUSSION

Very low level of hTFIID transcript has been demonstrated in HeLa cell using conventional RNA hybridization analysis.4) In normal human tissues, we examined the hTFIID gene expression by Northern blot using cDNA probe obtained from PCR products (data not shown). Detection of the levels of expression, however, was close to the lower limit of sensitivity of conventional hybridization. To improve the ability to detect low levels of hTFIID mRNA, we have devised a highly sensitive, specific and quantitative assay for measurement of the hTFIID gene expression.

An efficient quantitative assay for hTFIID mRNA based on PCR was developed in this study. Some PCR methods for the determination of mRNA have been reported as being highly specific and to give reliable results.13,15−17) The distinctive points of our procedure include the use of a small amount of total cellular RNA (1 µg), a random primer for cDNA synthesis, β2M mRNA as an internal control and calculation of \textsubscript{A\textsubscript{hTFIID}/A\textsubscript{β2M}}, from the efficiency R of hTFIID and β2M based on \textsuperscript{32}P-incorporation at different cycles of co-amplified PCR. In some tumors including SCLC, decreased expression of β2M and other HLA-related genes has been demonstrated.18) The expression of β2M was found in all the tissues and carcinomas in our co-amplified PCR, as has also been reported in other papers.17) The β2M transcript is thus shown to be usable as the internal control.

By the present co-amplified procedure, it was possible here for the first time to demonstrate the distribution of hTFIID transcript in normal human tissues. hTFIID expression appeared correlated to total mRNA initiation in the tissues. Moderate hTFIID expression was detected in tissues with active protein synthesis such as the liver, placenta and fetal lung.

Finally, the quantitative PCR procedure presented here can determine the amounts of low abundance specific mRNAs in very limited amounts of

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Relative value Relative to A\textsubscript{hTFIID}/A\textsubscript{β2M} total mRNA (%)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>0.2−0.4</td>
<td>2−4\times10\textsuperscript{-5}</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>&lt;0.02</td>
<td>&lt;0.2\times10\textsuperscript{-5}</td>
</tr>
<tr>
<td>Liver</td>
<td>0.8−1.5</td>
<td>8−15\times10\textsuperscript{-6}</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.4−0.7</td>
<td>4−7\times10\textsuperscript{-6}</td>
</tr>
<tr>
<td>Heart</td>
<td>0.1−0.2</td>
<td>1−2\times10\textsuperscript{-6}</td>
</tr>
<tr>
<td>Adult lung</td>
<td>&lt;0.02</td>
<td>&lt;0.2\times10\textsuperscript{-6}</td>
</tr>
<tr>
<td>Fetal lung</td>
<td>1.3−1.8</td>
<td>13−18\times10\textsuperscript{-6}</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.1−0.3</td>
<td>1−3\times10\textsuperscript{-6}</td>
</tr>
<tr>
<td>Placenta</td>
<td>1.6−2.5\textsuperscript{a}</td>
<td>16−25\times10\textsuperscript{-6}</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>0.8−1.2</td>
<td>8−12\times10\textsuperscript{-6}</td>
</tr>
<tr>
<td>Lu 134 BS</td>
<td>3.8−4.5\textsuperscript{b}</td>
<td>38−45\times10\textsuperscript{-6}</td>
</tr>
</tbody>
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\textsuperscript{a} Relative values were calculated from efficiencies, \textsubscript{R\textsubscript{hTFIID}}=48% and \textsubscript{R\textsubscript{β2M}}=38%, in 25 cycles PCR and triplicate runs.

\textsuperscript{b} Estimated values in 25 cycles PCR, almost the same as by quantitative analysis at different cycles (14−26 cycles).
total cellular RNA (1 μg). This technique can be used to study gene expression in a more extensive application, for example, clinical analysis and diagnosis of cancer, metabolic disorders and autoimmune diseases.

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REFERENCES

要旨
真核細胞において、転写開始因子の一つである TFIID は、遺伝子のプロモーター領域の TATA box と結合し、RNA ポリメラーゼ II により転写されるほとんどの遺伝子を制御している。Polymerase chain reaction (PCR) 法を用いたヒト TFIID (hTFIID) mRNA の高感度かつ特異的な定量化法を考案した。この定量化法の特徴は、微量の RNA (1 μg) を検出材料とすること、cDNA 合成にランダムプライマーを用いること、5'-ミクロプロックをコントロールとして用いることなど、特に異なったサイクル数の PCR を行い 32P の取り込み量より hTFIID 転写産物の相対量を算定することなどが挙げられる。この方法を用いて、ヒト正常組織における hTFIID の遺伝子発現の分布を初め明らかにするとともにその mRNA 量を定量した。その結果、肝、胎児肺および胎盤および中程度の hTFIID mRNA の発現が検出された。hTFIID 転写産物量および組織における転写開始される全 mRNA 量や蛋白合成量と相関すると考えられた。この PCR を用いた定量法は、特異的で高感度であり、より広範囲の遺伝子発現の検索に応用可能である。